Available online on 15.11.2014 at http://jddtonline.info
Journal of Drug Delivery and Therapeutics
Open access to Pharmaceutical and Medical research

REVIEW ARTICLE

BESIFLOXACINTHE FOURTH GENERATION FLUOROQUINOLONE: A REVIEW

***Singh Chhote Lal[1], Singh Amit [1], Kumar Sokindra[1], Majumdar D. K[2]**

[1]R. V. Northland Institute, Chithera, Dadri, Gautam Budh Nagar, Uttar Pradesh, India-203207

[2] Dr (Ex. Prof) Dipak Kanti Majumdar, Delhi Institute of Pharmaceutical Sciences and Research (DIPSAR), Pushp Vihar-III, M. B. Road, New Delhi, India, 110017,

ABSTRACT

Besifloxacin is fourth generation ophthalmic fluoroquinolone of synthetic origin, it was approved by the United States Food and Drug Administration (USFDA) in May 2009 for the treatment of bacterial conjunctivitis. It is only fluoroquinolone that has not been studied for systemic use; it was sold first in United States of America (USA) under the trade name of Besivance® ophthalmic suspension 0.6% formulated with Duracite®technology. This review article provides an overview of the pharmacology, efficacy and tolerability of besifloxacin suspension in the treatment of bacterial conjunctivitis, along with their different analytical techniques.Besifloxacin is a broad spectrum antibiotic active against ocular bacterial pathogen *Streptococcus pneumonia, Staphylococcus aureus, Staphylococcus epidermidis, Haemophilus influenza, Moraxella catarrhalis and Corynebacterium spp*.Conjunctival concentration of besifloxacin was reported and found to be C_{max}- 62.79µg/ml, AUC_{0-t} - 5569min.µg/ml, MIC_{90} <0.06-4µg/ml after single dose of besifloxacin ophthalmic suspension 0.6% in rabbit. In two randomised, double masked vehicle- control trial study reported the besifloxacin ophthalmic suspension was well tolerated and efficacious than vehicles.Besifloxacin was found to be well tolerated in clinical trial however common adverse effects were blurred vision (2.1%), bacterial conjunctivitis (1.8%), eye pain (1.5%) and non-specific bacterial conjunctivitis. Besifloxacin 0.6% ophthalmic suspension was reported to the well tolerated and efficacious in the treatment of bacterial conjunctivitis.There were several methods of analysis like UV, HPLC and microbiological for the quantification of besifloxacin.

Keywords: Besifloxacin, Moxifloxacin, Gatifloxacin, Bacterial conjunctivitis, HPLC Pharmacokinetic

INTRODUCTION

Bacterial conjunctivitis is an inflammation of the conjunctiva, characterised by persistent mucopurlent discharge and redness of the eye [1]. It is generally a self-limiting disease and usually does not cause permanent loss of vision or structural damage to eye. Topical anti-bacterial is used to overcome this disease [2, 3] .Bacterial conjunctivitis caused by those bacteria which infect the eye through various sources of contamination. The most common types of bacteria that cause bacterial conjunctivitis include *Streptococcus pneumonia, Staphylococcus aureus, Staphylococcus epidermidis, Haemophilus influenza, Moraxella catarrhalis and Corynebacterium spp* [4,5], *.Aerococcusviridans, CDC coryneform group G, Corynebacterium striatum, Haemophilus influenzae, Moraxella catarrhalis, Moraxella lacunata, Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus hominis, Staphylococcus lugdunensis, Staphylococcus warneri, Streptococcus mitis group, Streptococcus oralis, Streptococcus pneumoniae, Streptococcus salivary* [6].

Besifloxacin ophthalmic suspension 0.6%w/v was approved by US-FDA in 2009. The formulation uses Duracite® technology to increase the residence time of drug on theocular [7,8] .Besifloxacin is a novel fluoroquinolone antibiotic for ocular pathogens that are currently resistant to present fluoroquinolone antibiotics [9].

Besifloxacin (7-[(3R)-3- aminohexahydro -1H-azepin -1-y1] – 8 – choloro -1- cyclopropyl – 6-fluoro -1,4-dihydro -4-oxo-3-quinolinecarboxylic acid)–(figure 1)is a novel, chiral synthetic fluoroquinolone being developed by Bausch & Lomb for the topical treatment of ophthalmic infections. Structurally, besifloxacin has an N-1 cyclo-propyl group, which provides broad- spectrum activity against aerobic bacteria [10]. The main mechanism of action of besifloxacin is the inhibition of DNA gyrase and topoisomerase-IV enzyme that are involved in bacterial transcription replication and separation of chromosomal DNA during cell division [10]. This activity is enhanced by a C-8 chloride substituent in besifloxacin, which also exhibits improved activity against gram-positive bacteria relative to older fluoroquinolone while still remaining potent efficacy against anaerobic bacteria [11].

*Corresponding Author
Chhote Lal Singh
R. V. Northland Institute,
Chithera, Dadri, Gautam Budh Nagar,
Uttar Pradesh, India-203207
Phone no.- +91-7836062102, Fax No. +91-120-2666445
Email: chhotelal007@gmail.com

 ISSN: 2250-1177 CODEN (USA): JDDTAO

Cambau E et al. [12] investigated mechanism of action of BSF in *Streptococcus pneumoniae* and *Staphylococcus aureus* and found that BSF inhibit both enzyme DNAgyrase and topoisomerase-IV. Microbial data reports that besifloxacin have a relatively high potency and rapid bactericidal activity against conjunctivitis causing agents or bacteria [13]. However, this review article provides an overview of the pharmacologic and pharmacokinetic properties, clinical efficacy, and tolerability of besifloxacin 0.6% ophthalmic suspension along with HPLC and microbiological validation of besifloxacin bioassay.

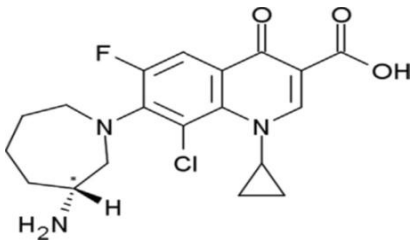

Figure 1: Chemical structure of besifloxacin.

METHODS OF CITATION

The reports related to pharmacology, efficacy, tolerability and validating methods like HPLC and by Bioassay. Microbiological assay of besifloxacin obtained through a search of PubMed, Medline, International Pharmaceutical Abstracts , Willey Library Online, Science Direct , Drug Review of R & D of besifloxacin and searching FDA website using the term besifloxacin, besivance, besix, bacterial conjunctivitis, HPLC methods of besifloxacin, microbiological assay of besifloxacin , mechanism action of besifloxacin, SAR of besifloxacin, safety and tolerability of ophthalmic suspension of besifloxacin, spectrum of besifloxacin, causative agents of bacterial conjunctivitis, ADR of besifloxacin, marketed products of besifloxacin HCL and dose administration of ophthalmic suspension of besifloxacin.

PHARMACOKINETIC PARAMETERS

Ward et al. [11] reported after instillation of a single dose of 0.6% ophthalmic suspension of besifloxacin in rabbit's eye and determined the ocular pharmacokinetic parameter of besifloxacin. These ocular pharmacokinetic parameter like $C_{max,}$ AUC, Mean residence time (MRT) of drug were studied in aqueous humours, tears and in plasma (Table 1).

Table 1: Pharmacokinetical parameters of besifloxacin ophthalmic suspension of 0.6% concentration after a single installation in rabbit ocular tissue [11]

Site	C_{max}(µg/ml)	AUC_{0-t} (min. µg/ml)	MRT (min.)
Tears	2756	194, 580	923
Conjunctiva	62.7900	5569	458
Aqueous humor	1.6920	125	422
Plasma	0.0198	2.55	NA

NA = Not available

Among the plasma, aqueous humours, conjunctiva and tears, very low concentration of besifloxacin (<10 µg/ml) was found in plasma after single dose, so it confirming minimal systemic exposure after ocular administration. The drug concentration in conjunctiva was more than plasma and aqueous humour and less than the concentration obtained in tears. The result of these simulation indicated that a thrice daily dosing regimen was sufficient to achieve conjunctival besifloxacin concentration of 2µg/g that were consistently above the MIC_{90} for most ophthalmologic.

Prokesh et al. [14] evaluated by administrating one drop of besifloxacin ophthalmic suspension 0.6% in each eye 3-times daily for 5-days with a final dosing on 6 th day in a subject with bacterial conjunctivitis. In another study a single topical ocular administration was given and found that the besifloxacin was rapidly absorbed (T_{max}=0.5 hour) and sustained concentration in tears of besifloxacin was found to be of <0.05 µg/ml that were maintained through 24 hours. The mean plasma concentration was 12-15 folds lower in human than in the experiment in rabbits and monkey. The in-vitro protein binding of besifloxacin in human plasma ranges from 38.5% - 44.0% (Table 2).

Table 2: Ocular pharmacokinetical parameters of besifloxacin ophthalmic suspension 0.6% in human tears [14]

Parameters	Value
C_{max}, mean(SD), µg/ml*	610(540)
T_{max}, min.	10
AUC_{0-24h}, µg.h/g*	1232
Elimination $t_{1/2}$,h*	~3.4
Systemic exposure, ng/ml†	<0.5

**Measured in tears of volunteers who received a single ocular administration of suspension.*
†Measured in plasma from patients who received besifloxacin ophthalmic suspension 3-times daily for 5-days.
C_{max}- Maximum concentration, T_{max}- Peak time, AUC - Area under curve

CLINICAL SAFETY AND EFFICACY

The clinical safety and efficacy of 0.6% ophthalmic suspension of besifloxacin in the treatment of bacterial conjunctivitis was studied and reported by Tepedino et al.[8], Karpeci et al. [15], McDonald MB et al. [16] Khimdas et al. [17]

Besifloxacin and Vehicle and others

Tepedino et al [8] , Karpeci et al.[15] and McDonald MB et al.[16] conducted a prospective, randomised, double masked, vehicle-controlled parallel group trial for a 5 days for evaluating the clinical safety, efficacy and microbiological inhibitory action of 0.6% ophthalmic suspension of besifloxacin in the treatment of bacterial conjunctivitis.

In their trial study 269 patients were selected for treatment (137- Besifloxacin, 132- Vehicle), however among them only 256 subjects completed the trial (134-Besifloxacin, 122- Vehicle) respectively. Where in the culture-confirmed study intent to treat population consisted of 118 patients (60-Besifloxacin 0.6% ophthalmic suspension and 58-Vehicle controlled) respectively. In this study the female subjects were (60.2%) in which white were 82.5%, with mean age 34.2 years[8, 15]. During this trial more patients were received 0.6% ophthalmic suspension of besifloxacin, as compared to vehicle. The clinical resolution of the baseline infection at visit -3 was found to be [44/60(73.3%) vs. 25/58 (43.1%) respectively, P<0.001]. The result as compared with vehicle in visit 3, the rate of bacterial eradication was also greater with besifloxacin suspension [53/60 (88.3%) vs. 35/58 (60.3%); P<0.001, (Table 3).

The adverse event was not differ significantly between the two groups [69/37 (50.4%)] and [70/32 (53.0%)].The most common ocular adverse effects were eye pain, blurred vision, eye irritation and visual acuity. However, Karpeci et al. [15] studied and reported that 0.6% ophthalmic suspension of besifloxacin, administered 3 times daily for 5 days was both efficacious and well tolerated as compared with vehicle in the treatment of patients with bacterial conjunctivitis. However, treatment with besifloxacin that was reported by Karpeci et al. were to be well tolerated, with no apparent differences compared with vehicle in terms of clinically significant changes in visual acuity or treatment-emergent events on bio microscopy or direct ophthalmoscopy. In addition, besifloxacin ophthalmic suspension 0.6% administered two- times daily for 3-days was clinically more effective than the vehicle alone for bacterial conjunctivitis.

Table: 3. Investigators rating of clinical signs and symptoms in the culture conformed to treat population.[15]

Clinical sign and symptom	Visit 2 (Day 4)		Visit3(Day 8)	
	Besifloxacin (n = 60)	Vehicle (n =56)	Besifloxacin (n = 60)	Vehicle (n = 56)
Ocular discharge	P = 0.008		P = 0.003	
Absent	36 (60)	26 (46.4)	54(90)	37(66.1)
Mild	20 (33.3)	18(32.1)	5(8.3)	15(26.8)
Moderate	4 (6.7)	11(19.6)	1(1.7)	3(5.4)
Severe	0	1(1.8)	0	1(1.8)
Bulbar conjunctival injection	P = 0.014		P = 0.013	
Normal	24 (40)	13 (23.2)	45(75)	30(53.6)
Trace	27(45)	30(53.6)	11(18.3)	21(37.5)
Moderate	8(13.3)	9(16.1)	3(5)	2(3.6)
Severe	1(1.7)	4(7.1)	1(1.7)	3(5.4)

**P value calculated by C.- Mantel Haenszel test.*

However, Tepedino et al. found 0.6% ophthalmic suspension of besifloxacin to be well tolerated in patients with bacterial conjunctivitis. Rate of absence of ocular discharge and eradication of the baseline infection were higher with 0.6% ophthalmic suspension of besifloxacin as compared to the vehicle. On the basis of earlier reporting Khimdas et al.[17]concluded that the besifloxacin is safe and efficacious for the treatment of bacterial conjunctivitis in patients without any bacterial resistance.

Malohotra et al.[18] also conducted randomized, multicentre, double masked, vehicle controlled, parallel group study on 518 selected subjects with mean age of ≥ 1 years. In trial study, patients were randomized into 2:1 to treatment with besifloxacin 0.6% ophthalmic suspension and vehicle for 7 days, through instilled one drop to infected eyes. They reported that besifloxacin 0.6% ophthalmic suspension is safe in patient to treat bacterial conjunctivitis infected eyes, when used three times daily for seven days.

COMPARISION WITHDIFFERENT FLUOUROQUINOLONEAND VEHICLE

McDonald MB et al.[16] conducted a prospective, randomized, double-masked, active-controlled, parallel-group, non-inferiority trial to compared the clinical and antimicrobial efficacy of 0.6% ophthalmic suspension of besifloxacin and 0.5% ophthalmic suspension of moxifloxacin, by administered both 3 times daily for 5 days, in the treatment of bacterial conjunctivitis. However, McDonald MB et al. found that 0.6% ophthalmic suspension of besifloxacin to be noninferior in tolerability and efficacy to 0.5% ophthalmic solution of moxifloxacin in the treatment of bacterial conjunctivitis. There were no significant differences found in rates of absence of ocular discharge and eradication of the baseline microbial infection between the two treatment groups.

Torkildsen G et al [19] conducted a trial to compared the pharmacokinetic parameter of besifloxacin, gatifloxacin and moxifloxacin and found that these fluoroquinolone were well tolerated in patients.

Silverstein et al. [20] reported that besifloxacin ophthalmic suspension 0.6% administered twice daily for 3 days leads significantly higher rates of clinical resolution and bacterial eradication as compared with vehicle and also was well tolerated, Table 4. The same was also reported by Karpeci et al.[15,] Tepedino et al.[8] and McDonald MB et al.[16,] (Table 5) but days of study was differ among them. Besifloxacin ophthalmic suspension was associated with significantly higher rates of clinical resolution compared with vehicle at visit-2 for infection with *H. Influenza, S.aureus, and S. epidermids* who significantly shows higher rates of bacterial eradication at each visit of infection [21-24] .In addition, besifloxacin MIC_{90} values against these species were found several folds lower than those of others comparator fluoroquinolone like moxifloxacin, gatifloxacin, ciprofloxacin, azithromycin, tobramycin, and vancomycin(Table 6).

Table: 4. Treatment emergent ocular adverse event.[20]

Study eyes, no (%)				All treated eyes no, (%)		
Variable	**Besifloxacin ophthalmic suspension (n = 94)**	**Vehicle (n = 157)**	**P**	**Besifloxacin ophthalmic suspension (n = 157)**	**Vehicle (n = 154)**	**p**
Total no. of AE	4	8		9	17	
Eyes with ≥ 1 AE	4(4.3)	8(8.2)	0.373	9(5.7)	17(11)	0.104
Specific AEs, no (%)						
Conjunctivitis bacterial	0	0	1	3(1.9)	5	0.499
Conjunctivitis	2(2.1)	3(3.1)	1	3(1.9)	4	0.721
Conjunctivitis, allergic	1(1.1)	1(1.0)	1	2(1.3)	1	1
Conjunctiva - l hypermia	0	1(1.0)	1	0	1	0.495
Instillation – site pain	1(1.1)	0	1	0	2	0.244
Corneal staining	0	1(1.0)	1	0	2	0.244

Table 5: Summary of phase III clinical efficacy studies of besifloxacin ophthalmic suspension 0.6% [8, 15, and 16]

Efficacy, n/N (%)				
Authors /study design	**Intervention**	**Clinical**	**Microbiologic**	**Adverse effect**
Karpecki et al [15]., MC, R, DM, VC, PG ,Prospective	Besifloxacin TID for 5 days (n= 137) Vehicle TID for 5 days (n= 132)	44/60 (73.3) 25/58 (43.1)	53/60 (88.3) 35/58 (60.3)	>5% eye pain (10.5% BSF, 6.9% vehicle),blurred vision ,eye irritation
Tepedino et [8]al.MC, R, DM, VC, PG, Prospective	Besifloxacin TID for 5 days (n= 473) Vehicle TID for 5 days (n= 484)	90/199 (45.2) 63/191 (33.0)	182/199 (91.5) 114/191 (59.7)	Blurred vision(1.2% and 2.2%), eye irritation(1.1% and 0.4%) , bacterial conjunctivitis (0.8% and 2.1%)
Mc Donald et al. [16]MC, R, DM, AC, PG, prospective, non-inferiority	Besifloxacin TID for 5 days (n= 582) moxifloxacin TID for 5 days (n= 579)	147/252 (58.3) 167/281 (59.4)	235/252 (93.3) 256/281 (91.1)	Blurred vision (1% and 0.5%), eye pain (0.6% and 1.1%), eye irritation (0.3% and 1.4%)

MC= Multi centre, R =Randomized, DM= Double masked, VC = Vehicle controlled, PG = Parallel group, AC = Active controlled.

Table 6: In-vitro activity of besifloxacin and other antibacterial agentagainst isolated bacterial pathogen. [21-24]

MIC_{90}, µg/ml							
Species	**B**	**M**	**G**	**C**	**A**	**T**	**V**
All species (n= 178)	0.25	0.25	0.5	2	128	16	ND
Gram positive (n=104)	0.25	0.5	0.5	2	256	32	2
Gram negative (n = 74)	0.06	0.06	0.06	0.06	4	2	ND
H.influenzae (n= 74)	0.06	0.06	0.03	0.015	4	2	ND
S.aureus (n =24)	0.5	2	2	8	128	1	1
S.epidermidis (n = 18)	0.5	4	2	64	>256	8	2
S. pneumoniae(n= 16)	0.12	0.25	0.5	2	0.25	32	0.5

B – Besifloxacin, M – Moxifloxacin, G – Gatifloxacin, C – Ciprofloxacin, A – Azithromycin, T – Tobramycin, V – Vancomycin

DOSAGE REGIMEN AND ADMINISTRATION

Besivance® [25] Besifloxacin ophthalmic suspension 0.6% w/v) is sterile topical ophthalmic eye drop of quinolone derivative. It is indicated for the treatment of bacterial conjunctivitis caused by susceptible isolates of bacteria.

Besivance® and Besix® two different marketed brands was formulated by 'Duracite' technology containing 0.6% sterile ophthalmic suspension of besifloxacin which are available in 5 ml of sterile packing. Each ml of 0.6% suspension contains 6.63 mg of besifloxacin hydrochloride equivalent to 6 mg of the drug. The suggested dose is one drop to the bacterial infected eye, 3 times daily for 7 days [25] . Besifloxacin is only recommended for topical ophthalmic use and should not be injected sub conjunctively, nor should it be introduced directly into the anterior chamber of the eye. Patients who have signs and symptoms of bacterial conjunctivitis should not wear contact lenses. During the time of administration invert the close bottle, as well as suspension bottle must be shake once before use.

ANALYSIS OF BESIFLOXACIN

Arnold et al. [26] quantitatively determined besifloxacin in human tear by liquid chromatography by using sparfloxacin as the internal standard. Calibration standard were prepared by spiking schirmer strips containing the artificial tear solution with known concentration of besifloxacin. In this study tear sample was collected from 64 healthy subject following topical ocular administration of 0.6% besifloxacin hydrochloride in both eyes with the aid of spiking schirmer strips and then analyzed by LC/MS/MS. This artificial tear strip was weighed before and after tear collection and the test strip were store at -20^0C until the sample were analyzed. However, the reported quantitative analysis of besifloxacin in human tear sample was found to maximum level was 610±540 µg/g after topical administration of 0.6% ophthalmic suspension and tear sample were collected and analyzed 24 hours after dosing, the method was précised and economical with short run time 4 minutes and applied for topical ocular pharmacokinetic of besifloxacin.

Costa C. N. et al. [27] reported the development and validation of microbiological assay method that was compared with high performance liquid chromatography (HPLC) method. The study was based on the inhibitory effects of besifloxacin on the strain of *Staphylococcus epidermidis* (ATCC1228). The standardization procedure was based on the protocol of Brazilian Pharmacopoeia-2010and USP-2009 (Table 7).In themicrobiological study reported by O,Brien TP [28] shows a relatively high potency and rapid bactericidal activity for besifloxacin against ocular pathogen especially resistant staphylococcal species. All the HPLC methods were validated in their study according to USP-34, 2011 and International conference on Harmonization guidelines (ICH), 2005[29.]

Table 7: Evaluation by HPLC and microbiological method of BSF ophthalmic suspension after exposure to UVA light.[27]

Time (min.)	BSF ophthalmic suspension %	
	Microbiological assay	**HPLC**
0	100	100
60	91.5	91
120	81.4	78
180	74.7	66
240	69.1	60
300	60.7	53

CONCLUSION

Besifloxacin is a new broad-spectrum, bactericidal, topical ophthalmic fluoroquinolone approved for the treatment of bacterial conjunctivitis. Besifloxacin ophthalmic suspension was well tolerated in patients with a minimal ocular adverse effect. In addition to its broad spectrum of activity, clinical safety, efficacy and good tolerability of besifloxacin having also anti-inflammatory activity through inhibition of pro-inflammatory cytokines [30] and also through inhibition of the nuclear factor KB and mitogen activated protein kinase pathway. The low minimum inhibitory concentration and high attainment of pharmacodynamics target with besifloxacin may contribute to a lower risk for the emergence for the bacterial resistance, although further studies are required. Fluoroquinolone, particularly besifloxacin, gatifloxacin and moxifloxacin have become important treatment option for common

ocular bacterial infection due to their broad spectrum bactericidal activity and low toxicity. Besifloxacin ophthalmic suspension 0.6% provides safe and efficacious treatment for bacterial conjunctivitis. The factor leading to bacterial resistance diminished, which always besifloxacin to be a favorable treatment option. We also summarized the different Pharmacokinetical data and their analysis with different analytical methods like microbiological and HPLC etc.

ACKNOWLEDGEMENT

The authors would like to grateful of the authorities of R.V. Northland Institute, Greater Noida, UP, India for providing required facilities to carry out the proposed review work.

CONFLICT OF INTEREST

The authors have no conflicts of interest with regards to the content of this review article.

REFERENCES

1. Hovding G. Acute bacterial conjunctivitis: Acta Opthalmol, 2008, 86, 5-17.
2. Tarabishy AB, Jeng BH. Bacterial conjunctivitis: A review for internists: Cleve Clin J Med 2008, 75, 507-512.
3. Chung CW, Cohen Ej. Eye Disorders: Bacterial conjunctivitis: West J Med, 2000, 173, 202-205.
4. Sheikh A, Hurwitz B. Antibiotics versus placebo for acute bacterial conjunctivitis: Cochrane Database Syst Rev, 2006, (2), CD001211.
5. Rose PW, Harden A, et al. Chloramphenicol treatment for acute infective Conjunctivitis in children in primary care: A randomized double blind placebo – Controlled Trial : Lancet. 2005, 366, 37-43.
6. Dermatologic and Ophthalmic Drugs advisory committee meeting briefing document. Besifloxacin 0.6% w/v ophthalmic suspension for the treatment of Bacterial Conjunctivitis. www.fda.gov/ohrms/dockets/ac/08/briefing/2008 - 4397b1-02-BauschLomb.pdf.AccessedAug. 2009
7. US Dept. of Health and Human Services. NDA 22-308. NDA approval.http://www.acessdata.fda.gov/drugsatfdadocs/appletter /2009/022308s000Itr pdf.Accessed Aug. 2009.
8. Tepedino ME, Heller WH, Usner DW, et al. Phase III Efficacy and safety study of besifloxacin ophthalmic suspension 0.6% in the treatment of Bacterial Conjunctivitis: Curr Med Res Opin.2009, 25, 1159-1169.
9. Besivance (package inserts). Tampa, Fla: Bausch and Lomb Incorporated, 2009.
10. Zhang JZ, Ward KW. Besifloxacin a Novel Fluoroquinolone antimicrobial agent, exhibits potent inhibition of pro-inflammatory cytokinines in Human THP-1 Monocytes: JAntimicrob Chemother. 2008, 61, 111-116.
11. Ward KW et al. Non-clinical pharmodynamics, pharmacokinetic, safety of BOL- 303224-A, and a novel fluoroquinolone antimicrobial agent for topical ophthalmic use : J Oculpharmacol Ther.2007, 23, 243-256.
12. Cambau E, Matrat S, et al. Target specificity of the new fluoroquinolone besifloxacin S. pneumoniae , S. aureus ,E. coli: J Antimicrob Chemother.2009, 63, 443-450.
13. Hass W, Piller CM, Zurenko GE , et al. Besifloxacin , a novel fluoroquinolone , has broad spectrum In vitro activity against Aerobic and An – aerobic Bacteria : Antimicrob Agents Chemother .2009, 53, 3552-3560.
14. Prokesh JW et al. Ocular pharmacokinetics of besifloxacin following topical administration to Rabbits, Monkeys, and Humans : Ocular Pharmacol Ther. 2009, 25, 335-334
15. Karpeci P et al. Besifloxacin ophthalmic suspension 0.6% in Patients with bacterial conjunctivitis: A multicenter, prospective, randomized, double masked, vehicle – controlled, 5-days efficacy and safety study: Clin Ther.2009, 31, 514-526.
16. MC Donald MB et al. Efficacy and safety of besifloxacin ophthalmic suspension 0.6% compared with Moxifloxacin ophthalmic suspension 0.5% for treating Bacterial Conjunctivitis : Ophthalmology 2009, 116, 1615-1623.
17. Khimdas S,VKL. Besifloxacin ophthalmic suspension : emerging evidence of its therapeutic value in bacterial conjunctivitis. Opthalmol Eye dis.2011 ;7-12
18. R Malohtra, Gearinger LS, Morris TW, Haas W : The safety of Besifloxacin ophthalmic suspension 0.6% used Three Times Daily in the treatment of bacterial conjunctivitis: Palm Beach, 2010, 18-21.
19. G. Torkildsen, J W, Proksch, A Shapiro, S K, T L Comstock : Clin. Opthalmol. 4 2010, 331 – 341.
20. Bruce E. Silverstein et al. Efficacy and tolerability of besifloxacin ophthalmic suspension 0.6% administered twice daily for 3 days in the treatment of bacterial conjunctivitis: A multicenter, randomized, double masked, vehicle –controlled, parallel group study in adults and children, clin. Ther. /33, 2011.
21. Chang JS, Flynn HW J, Miller D, Alfonso EC. Evaluation of in vitro effectiveness of besifloxacin against Staphylococcus Epidermidis. Presented at: Annual meeting of the association for research in vision and ophthalmology, May2-6, 2010 Fort Lauderdale, Fla.
22. Miller D. In Vitro Evaluation of Besifloxacin against Ciprofloxacin and Moxifloxacin resistant /Susceptible Staphylococcal isolate from South Florida. Presented at 5th International Conference on Ocular Infection; February 18-21, 2010; Palm Beach, Fla.
23. Gearinger LS, Haas W, Morris TW. In Vitro activity of besifloxacin, ciprofloxacin, gatifloxacin, levofloxacin and moxifloxacin against ciprofloxacin – Resistant ocular Staphylococcus aureus and Staphylococcus epidermidis. Presented 5th International Conference on Ocular Infection; February 18-21, 2010; Palm Beach, Fla.
24. Haas W, Zurenko GE, Lee, et al. Activity of Besifloxacin and competitors against Ciprofloxacin Resistant Staphylococcus aureus and staphylococcus epidermidis Ocular Isolate from 2005-2008.presented at:3rd Congress of the Federation of European Microbiologists ; June 28- July 2,2009; Goteborg, Sweden.
25. Besivance Ophthalmic Suspension 0.6% [Canadian product monograph].Canada; Bausch & Lomb, Inc., 2009.
26. Dana R. Arnold et al. Quantitative determination of Besifloxacin, a novel Fluoroquinolone antimicrobial agent, in human tears by Liquid Chromatography – tandem Mass Spectrometry; Journal of chromatography B 867, 2008, 105-110
27. Marcia C.N.Costa et al. Quantitative evaluation of besifloxacin ophthalmic suspension by HPLC, application to bioassay method and Cytotoxicity Studies, Talanta 2013.
28. Terrence P. O'Brien. Adv. ther 29 (2012) 473-490
29. ICH Harmonized Tripartite Guidelines; Validation of analytical procedure, Methodology ICH Steering Committee. Surca, 2005.
30. Zhang JZ, Cavet ME, Ward KW. Anti- inflammatory effects of besifloxacin, a novel fluoroquinolone, in Primary Human Corneal Epithelial Cells: Curr Eye Res.2008, 33, 923-932

.

